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Effects of molecular structures on the olfactory responses of phospholipid membranes to four alcohols

Qingzhong Zhou [a,*], Yanan Yang [a], Zhikuan Chen [a], Anna Sun [b]

[a] *College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, People's Republic of China*
[b] *Institute of Semiconductors, Chinese Academy of Sciences, People's Republic of China*

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Abstract

In order to understand the relationship between phospholipid molecular structures and their olfactory responses to odorants, we designed and synthesized four phosphatidylcholine analogues with different long hydrocarbon (CH) chains and selected three natural phospholipids with different head-groups. By using interdigital electrodes (IEs) as olfactory sensors (OSs), we measured the responses of the IEs coated with these seven different lipid membranes to four alcohol vapors in a gas flow system. The IEs voltage changes were recorded and the voltage-relative saturate vapor pressure (V-P/P°) curves were also plotted. It was found that with a methyl ($-CH_3$) placed at the C-8 position in the 18-carbon chain, the olfactory responses could be improved about ten times and with conjugated double bonds (C=C) in the long chains, the sensitivity could be increased by $3 \sim 4$ orders of magnitude. As to head-groups, choline is preferred over ethanolamine and serine in phospholipid structures in terms of high olfactory sensitivity. These results are expected to be useful in further designing and manufacturing lipid-mimicking OSs.

Keywords: Olfactory sensor; Interdigital electrode; Hydrocarbon chain; Head-group; Voltage response

Abbreviations: IE, interdigital electrode; OS, olfactory sensor; CH, hydrocarbon; 2-(8-Me)DSPC, 1-stearoyl-2-(8-methyl)stearoyl-sn-glycero-3-phosphatidylcholine; 1,2(8-Me)DSPC, 1,2-di-(8-methylstearoyl)-sn-glycero-3-phosphatidylcholine; DEPC, 1,2-dielaeostearoyl-sn-glycero-3-phosphatidylcholine; LPC, lysophosphatidylcholine; PC, L-α phosphatidylcholine; PE, L-α phosphatidylethanolamine; PS, L-α phosphatidylserine; MeOH, methanol; EtOH, ethanol; PrOH, propanol; iso-PrOH, iso-propanol; Tc, temperature of phase transition; DSC, differential scanning calorimetry; V-P/P°, voltage-relative saturate vapor pressure.

* Corresponding author. Tel.: +86 1 067291869; fax: +86 1 062574819; e-mail: postmaster@chemms.chem.pku.edu.cn

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1. Introduction

Olfaction is a specific chemical sense of animals to odorants. Its sensitivity and resolution power are so remarkable that they can reach molecular level. In 1987, Kurihara K. et al. put forward the olfactory proposal of lipid-adsorption and the model of interface potential changes (Nomura and Kurihara, 1987a,b). They suggested that olfactory stimulation initially involve odorant molecules being adsorbed to a lipid layer of olfactory cell membranes. This causes a potential change of the membrane and then generates a nervous impulse. Olfactory discrimination is due to the differences of lipid components and structures in each olfactory cell membrane that produce different membrane potentials to different kinds of odorant molecules (Nomura and Kurihara, 1987a; Entomoto et al., 1991). As a result, it has been found that lipids could be used as sensitive membranes for olfactory and taste sensors (Okahata et al., 1989; Tamiya et al., 1990; Nakamoto et al., 1991).

Based on the hydrophobic adsorption or solution in the hydrophobic area, odorant molecules can affect the potential and other electronic properties of olfactory cell membranes by changing the conformation of membrane molecules, the liquid crystallinity (ordering and mobility) and the polar head-group charges or dipolar ions' strength and orientation (Nomura and Kurihara, 1987b). Sun et al. had made olfactory sensors (OSs) by using interdigital electrodes (IEs) coated with phosphatidylcholine and similar chemicals as sensitive membranes. The characteristics of these sensors are quite similar to the human olfactory sense (Sun et al., 1994, 1995). In this paper, in order to have a better understanding about the relationship between phospholipid molecular structures and their olfactory responses to odorants, we designed and synthesized (Charp et al., 1988; Menger et al., 1988a; Zhou et al., 1988; Zhou, 1992) four phosphatidylcholine analogues, 2-(8-Me)DSPC, 1,2(8-Me)DSPC, DEPC and LPC. We also selected three natural phospholipids, PC, PE and PS as objects. The four phosphatidylcholine analogues have different long hydrocarbon (CH) chains, for example, there is a methyl substituent at the C-8 position in the 18 carbon chain or there are conjugated double bonds in the long chain. The three natural phospholipids have different head-groups which are choline, ethanolamine and serine. These seven lipids were coated on the IEs by using the method described previously (Cai et al., 1993; Sun et al., 1995). In a gas flow system, the IEs gave the responses to four alcohol (methanol, ethanol, n-propanol and iso-propanol) vapors at different relative saturate vapor pressure. The voltage responses of IEs were then detected and the voltage-relative saturate vapor pressure (V-P/P°) curves were given. Our results show that the olfactory responses to odorants vary greatly with lipid molecular structures.

2. Experiment

2.1. Lipids

Four phosphatidylcholine analogues (1)–(4) and three natural phospholipids (5)–(7) are as follows:

1. 1-stearoyl-2-(8-methyl)stearoyl-sn-glycero-3-phosphatidylcholine (2-(8-Me)DSPC for short), a methyl at C-8 of one long CH chain.
2. 1,2-di-(8-methylstearoyl)-sn-glycero-3-phosphatidylcholine (1,2(8-Me)DSPC), a methyl at C-8 in both chains.
3. 1,2-dielaeostearoyl-sn-glycero-3-phosphatidylcholine (DEPC), conjugated double bonds in both chains.
4. lysophosphatidylcholine (LPC), with only one long chain.
5. L-α phosphatidylcholine (PC), from egg yolk, purchased from Sigma Co., purity approx., 99%, with choline as head-group
6. L-α phosphatidylethanolamine (PE), purchased from Sigma Co., with ethanolamine as head-group
7. L-α phosphatidylserine (PS), from bovine brain, purchased from Sigma Co., with serine as head-group

Fig. 1 shows the structures of the seven phospholipids.

2.2. The syntheses of the four analogues

2.2.1. The synthesis scheme of 8-methyl stearic acid

Fig. 2 demonstrates the synthesis scheme of 8-methyl stearic acid. The conditions of every reaction step:

1. EtOH, $EtO_2C(CH_2)_6CO_2Et$/Conc. HCl, dibutylether/heating
2. $SOCl_2$
3. $[CH_3(CH_2)_9]_2Cd$/benzene
4. CH_3MgBr/diethyl ether
5. PBr_3/Pyridine/heating
6. H_2/PtO_2
7. KOH/H_2O–EtOH

2.2.2. The syntheses scheme of the four analogues

Fig. 3 demonstrates the syntheses scheme of the four analogues. The conditions of every reaction step:

1. $Et_4N^+OH^-$, ether/$CdCl_2$, EtOH
2. Snake venom, $CaCl_2$/EtOH, Et_2O, H_2O
3. 8-methyl stearic acid, DCC, DMAP/$CHCl_3$ or elaeostearic acid, DCC, DMAP/$CHCL_3$

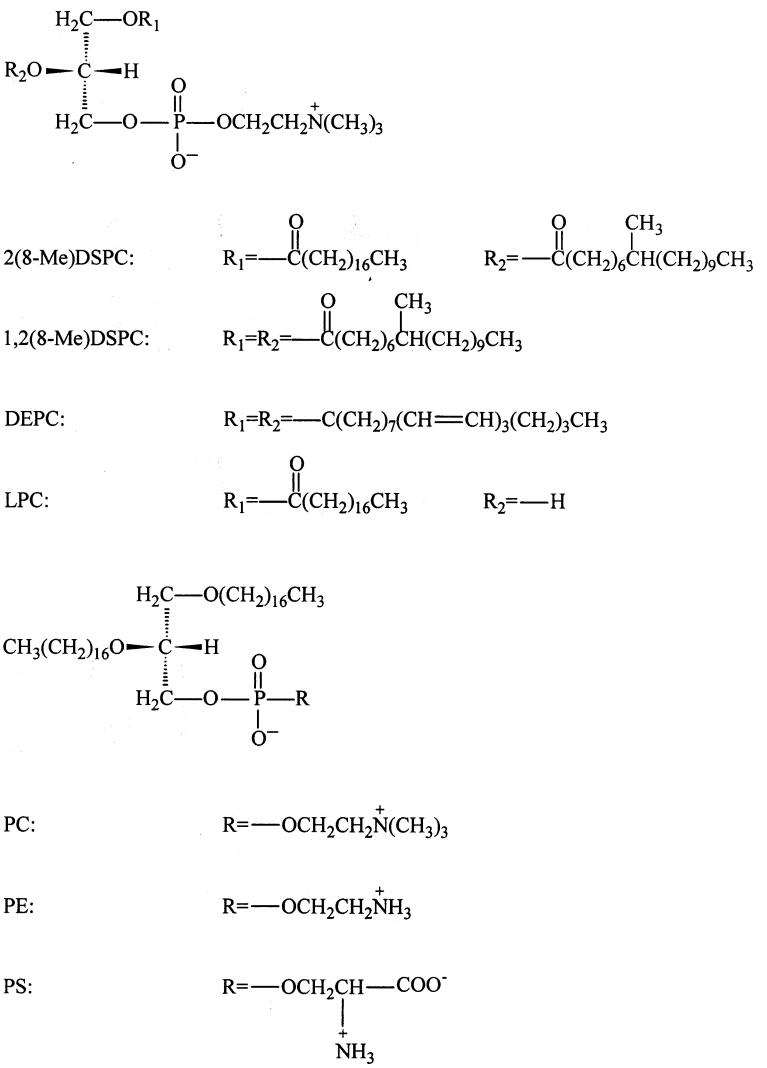

Fig. 1. Molecular structures of 2-(8-Me)DSPC, 1,2(8-Me)DSPC, DEPC, LPC, PC, PE and PS.

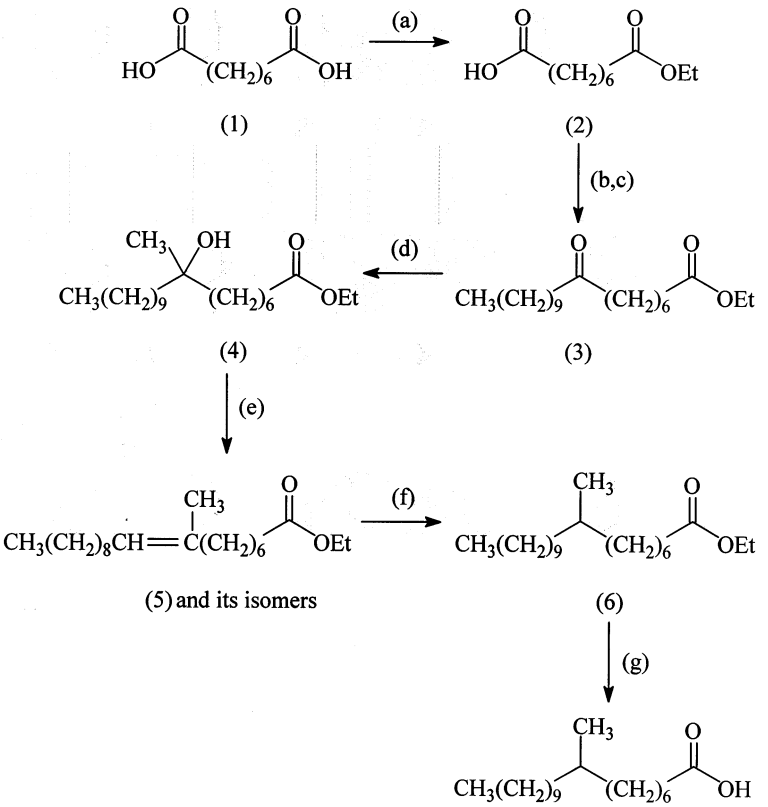

Fig. 2. Synthesis scheme of 8-methyl stearic acid.

4. 8-methylstearic anhydride, DMAP/$CHCl_3$

2.3. Measurements of membrane properties

The phospholipid was dissolved in organic solvent, commonly a mixture of chloroform and ethanol (1:3 v/v). Then it was coated on an IE to form the olfactory sensitive membrane by self-assembly. The coating procedure was as follows. A 2 μl solution was added to the IE chip by microsyringe at room temperature. After evaporation of the solvent and storage for 2 h, the IE was ready for use. The IE was suspended in a gas flow system. In the system, the carrier gas saturated by an odorant vapor at constant temperature (25.0 ± 0.5°C) had been diluted to different relative vapor pressures with dry air. The voltage changes of IE to odorants were measured. The V-P/P° curves were plotted.

The membrane structures were measured by small angle X-ray diffraction and the temperature of phase transition (Tc) was obtained by differential scanning calorimetry (DSC).

3. Results and discussion

According to the measurements described in Section 2, we found that the IEs have no voltage change to alcohol vapors without the phospholipid membranes. After having been coated with these phospholipid membranes, they can give similar V-P/P° curves which all belong to adsorption isotherm III. At a low concentration of the odorant vapor, there is only adsorption, which means that alcohol molecules only interact with lipid molecules. At a high concentration, adsorption co-exists with condensation, in other words, alco-

hol molecules interact not only with lipid molecules but also with themselves. The V-P/P° curves shown in Fig. 4 indicate 1,2(8-Me)DSPC, 2-(8-Me)DSPC, LPC, PC and DEPC membranes' responses to three alcohol vapors, ethanol (EtOH), propanol (PrOH) and iso-propanol (iso-PrOH).

In Fig. 4, these five lipid membranes have the same olfactory response pattern. All obey Steven's Law. It indicates that 1,2(8-Me)DSPC, 2-(8-Me)DSPC, PC and DEPC membranes have the same order of the response strength to the three alcohols. The order is: DEPC $\gg$ 1,2(8-Me)DSPC $>$ 2-(8-Me)DSPC $>$ PC. However, LPC is more special, it has not a fixed position in the order. With only one long CH chain in the molecule, the LPC membrane is not stable and this flexibility can produce a special response pattern. In order to demonstrate the results more clearly, Table 1 gives the voltage responses of these five kinds of membranes to methanol and ethanol at 50% relative saturate vapor pressure (25°C, P/P°).

From Fig. 4 and Table 1, the membrane responses increased greatly with a methyl substituent or conjugated double bonds in the CH chains of phospholipids. As to 1,2(8-Me)DSPC and 2-(8-Me)DSPC with methyl substituents in the long CH chains, their voltage responses are 10–20 times higher than that of PC. As to DEPC with three conjugated double bonds in the long chains, the response has been improved four orders of magnitude compared with that of PC. These results are related to the effects of the methyl and conjugated double bonds on the conformation and liquid crystallinity of lipid membranes.

When all the C–C bonds of long CH chains of fatty acid are at the all-*trans* position, the conformation is more stable and the carbon chain is in

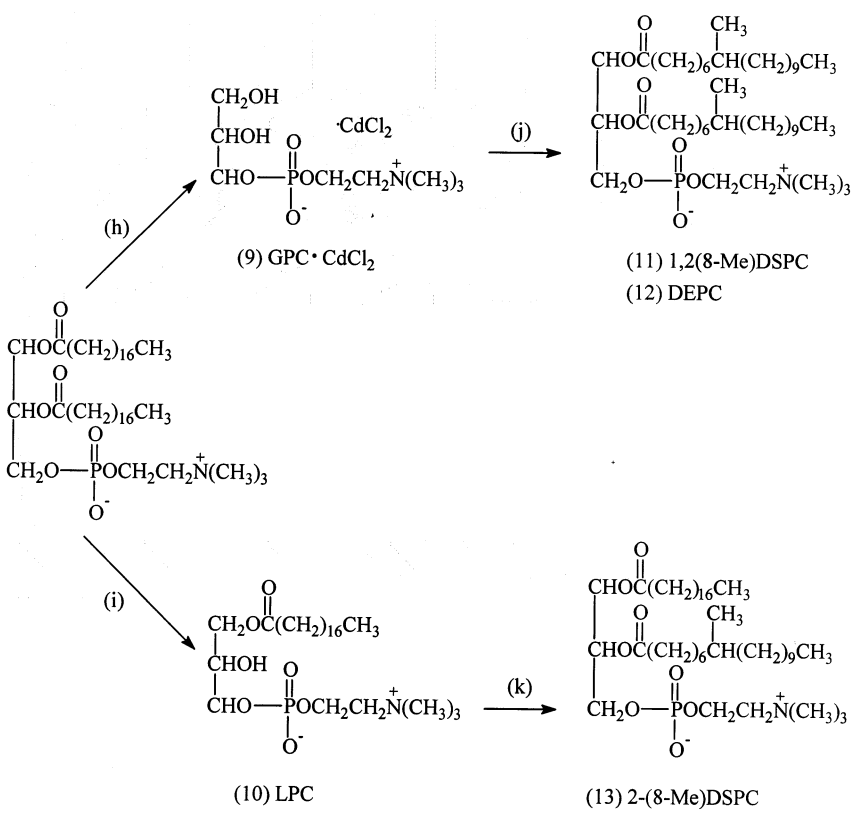

Fig. 3. Syntheses scheme of 2-(8-Me)DSPC, 1,2(8-Me)DSPC, DEPC and LPC.

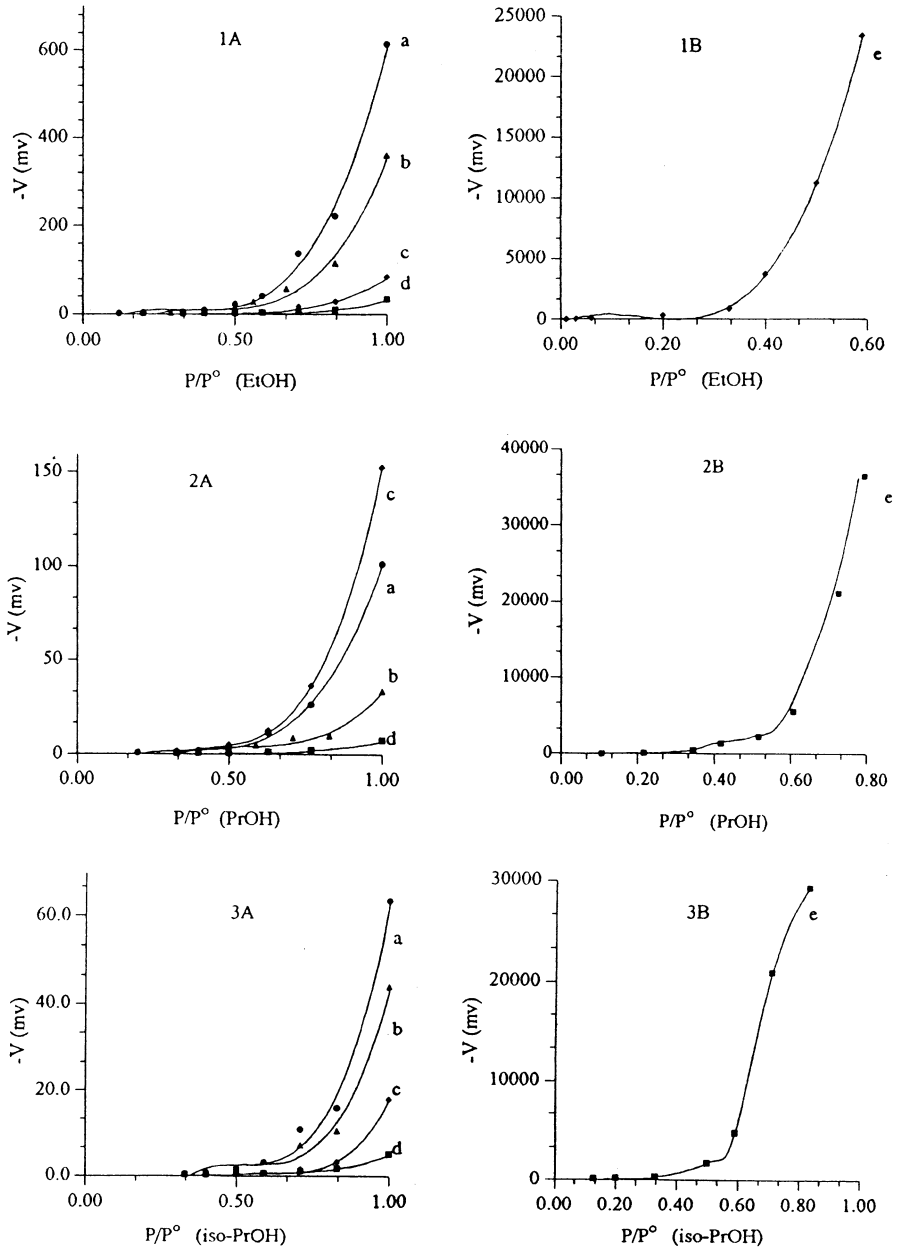

Fig. 4. Voltage-relative saturate vapor pressure (V-P/P°) curves to EtOH, PrOH and iso-PrOH vapors (a): 1,2(8-Me)DSPC; (b): 2-(8-Me)DSPC; (c): LPC; (d): PC; and (e): DEPC.

the shape of sawteeth in a plane. When there is a methyl group in the chain, the conformation tends to convert from *trans* to *gauche* (Menger et al., 1988b). For instance, see Fig. 5A, with a methyl at C-8 position of stearic acid, the linearity of the latter half chain can be reestablished through a second gauche to give a so-called 2gl kink, which means that at the methyl substituted carbon, the C–C bond rotates by 120° (g^+), the next C–C bond is *trans* (t) and the third C–C bond gives

Table 1
Voltage responses (mv) of 2-(8-Me)DSPC, 1,2(8-Me)DSPC, DEPC, LPC and PC membranes to 50% relative saturate vapor pressure of EtOH and MeOH

	1,2(8-Me)DSPC	2-(8-Me)DSPC	LPC	DEPC	PC
50% EtOH	20.2	13.4	1.4	11 300	0.8
50% MeOH	130.0	102.3	3.2	>40 000	5.1

another rotation by $-120°$ (g^-). Consequently, the overall chain length decreases 1.3 Å and the molecule volume increases 25–50 $Å^3$ (Menger et al., 1988a). As a unit which can induce disorder in the molecule, the methyl permits a degree of conformational flexibility at the site of disruption. The result of calculations reveals that the molecular across area of 8-methylstearic acid increases 50%. Another consequence of introducing methyl at C-8 position is that it can stabilize the flexible bend at the center of the alkyl chain. Accordingly the segment of the long alkyl chain nearer to the head group lies at a 30° angle to the membrane surface and the terminal part is perpendicular to the surface. This induces a bend at the center of the chain (without any substituent at the center, the bend is not stable). This central bend provides the second half of the chain about 12% more space in which to maneuver. More space can benefit the adsorption of membranes to odorants and then increase the olfactory sensitivity.

Elaeostearic acid is *cis*, *trans*, *trans*-9,11,13-octadectrienoic acid. Double bonds also make the conformation of long CH chain change and stabilize the center bend. Fig. 5B shows the conformation of elaeostearic acid. Double bonds are rigid and the conjugated system averages the relevant single and double bonds. The π-electrons between carbon

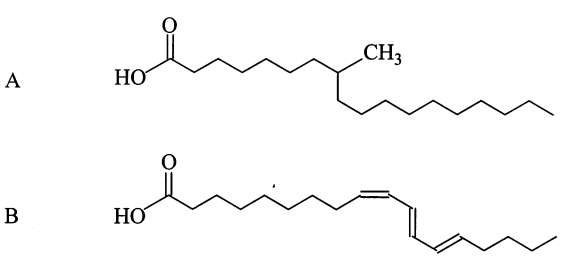

Fig. 5. Conformations of two kinds of CH chains. A: 8-methyl stearic acid, B: *cis*, *trans*, *trans*-9,11,13-octadectrienoic acid.

atoms are overlapped and easily polarized, which can produce a stronger electric signal. So double bonds can make a stable and rigid conformation and impart a bow-shaped molecule, which enhances the adsorption of odorants.

The introduction of substituents and double bonds induces the change of membrane liquid crystallinity, increases the mobility and decreases the phase transition temperature. The membrane tends to change from gel to liquid crystal phase. All these results agree with the measurements of DSC, Tc: PC: 54.8°C; 2-(8-Me)DSPC: 18.6°C; 1,2(8-Me)DSPC < 0°C. Therefore, increasing the membrane mobility can enhance olfactory sensitivity.

As to head-groups, PC, PE and PS have different olfactory responses to odorants. From Table 2, PE and PS only have a small response to methanol and ethanol at high concentration (80% more). It indicates that choline as the head-group of PC is essential. It is a zwitter ion in which a positive charge balances a negative charge. The positive and negative charges in the head-group benefit the change of membrane electronic properties. The three methyls in the choline provide a more extensive conformation to the head of the molecule which in turn provides more space for the hydrophobic chains to maneuver. This structure is important for the entry of odorants into membrane and for the inducement of the change in electronic properties. Fig. 6 shows the head-group structure of PC.

The membrane structure was measured with small angle X-ray diffraction. It shows that the adsorption peak lay at 40–60 Å, which is lower than the thickness of 60–75 Å of lipid bilayer. Here we can conclude that the lipid bilayer is formed through self-assembly in inclining or non-perpendicular packing. The membrane structure is a laminar accumulation in two dimensions.

Table 2
Voltage responses (mv) of PC, PE and PS to 80% relative saturate vapor pressure of EtOH and MeOH

	PC	PS	PE
80% EtOH	7.2	0	0.4
80% MeOH	45.7	6.0	2.4

4. Conclusion

1. The membranes of PC, 2-(8-Me)DSPC, 1,2(8-Me)DSPC, DEPC and LPC by self-assembly have a similar olfactory response pattern.
2. The structural differences of two long CH chains in phospholipids, which include their conformation and polarity, influence the membrane order and mobility. This can result in a different response sensibility of membranes to odorants. Methyl and conjugated double bonds introduced in the long chains can improve the sensitivity substantially.
3. The head-group structure of the phospholipid is very important to its olfactory responses. Among the three head-groups studied, choline-with a zwitter ion structure and a big trimethyl ammonium group volume—is the most effective.
4. The membranes of four phosphatidylcholine analogues synthesized can have a higher olfactory response than that of natural materials. This shows that the olfactory response function of membranes is related to the structures of the membrane molecules. Such a result is useful in further designing and manufacturing lipid-mimicking OSs.

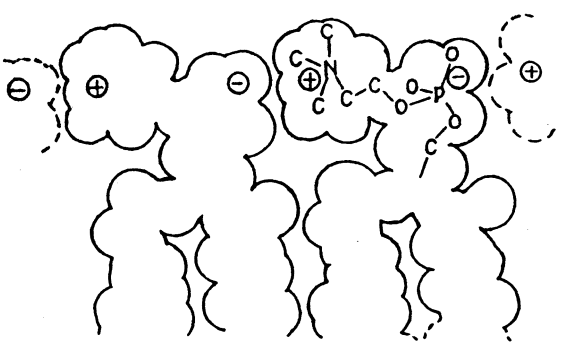

Fig. 6. The head-group arrangement of phosphatidylcholine membranes.

Acknowledgements

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